SHORT COMMUNICATION

EXPERIMENTAL INFECTION OF A FRESHWATER SNAIL, *POTAMOPYRGUS ANTIPODARUM*, WITH A DIGENETIC TREMATODE, *MICROPHALLUS* SP.

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ABSTRACT

Lively, C.M. & McKenzie, J.C. (1991). Experimental infection of a freshwater snail, *Potamopyrgus antipodarum*, with a digenetic trematode, *Microphallus* sp. *New Zealand Natural Sciences* 18: 59-62.

The life cycle of the digenetic trematode *Microphallus* sp. was completed in the laboratory, using a mouse as the definitive host. Metacercariae from this trematode were collected from the intermediate host, the freshwater snail *Potamopyrgus antipodarum*, and orally inoculated into the mouse. Field collections of *P. antipodarum* from two different populations were then exposed to the faecal pellets produced by this mouse over a 5 day period following inoculation. After 3 months, the levels of infection in the experimental snails were significantly greater than for snails held in a control aquarium. Hence, mice may be substituted for the normal definitive host of *Microphallus* (ducks), and may be used to complete the life cycle of this trematode under experimental conditions.

KEYWORDS: Prosobranchia - *Potamopyrgus antipodarum* - Microphallidae - *Microphallus* - experimental infection - life cycle.

INTRODUCTION

The freshwater snail *Potamopyrgus antipodarum* is the most abundant gastropod in most New Zealand lakes and streams. Some populations of this prosobranch gastropod contain only asexual (ameiotic) females, whereas other populations contain males and, presumably, at least some sexual females (Winterbourn 1970, Lively 1987, Phillips and Lambert 1989). Populations in which males are rare or absent also tend to have lower levels of infection by castrating trematode larvae (Digenea). This suggests that asexual reproduction has been favoured where the risk of parasitism is low (due to the advantage of not producing males [Maynard Smith 1978]) and that sexual reproduction is favoured where the risk of parasitism is high (Lively 1987). The advantage of sexual reproduction in heavily parasitised populations might stem from the production of rare genotypes, which are expected to be more likely to escape parasites in a coevolutionary host-parasite game (Levin 1975, Jaenike 1978, Bremermann 1980, Hamilton 1980).

The most common of the digenetic trematode larvae infecting *P. antipodarum* is a presently undescribed species of *Microphallus* (S. Deblock, Faculté de Pharmacie, Lille, France, pers. comm.). The adult stage of this microphallid parasite occurs naturally in the intestines of ducks and wading birds (Lively and McKenzie, pers. obs.). Eggs produced by these worms are passed with the faeces, and hatch following ingestion by *P. antipodarum*. Successful infections result in the production of several hundred to several thousand blastocercariae, depending on the size of the snail (Winterbourn 1974, Lively 1987). These tailless blastocercariae then encyst and form metacercariae in the same snail. The purpose of the present study was to determine whether the life cycle of *Microphallus* sp. could be completed in the labora-

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tory using mice as the definitive host. Mice have been successfully used to rear another trematode of the same genus, *Microphallus pygmaeus* (Ahmad *et al.* 1986, Ahmad and James 1987), but it was not known whether the eggs produced were viable and infective to its snail host (*Littorina saxatilis tenebrosa*). Completion of the life cycle in the laboratory would greatly facilitate the experimental study of the effects of *Microphallus* sp. on the reproductive strategy and genetic diversity of *P. antipodarum*, and would add a valuable system for the study of trematode life cycles, in general.

MATERIALS AND METHODS

P. antipodarum was collected from two lakes on opposite sides of the Southern Alps of New Zealand's South Island: Lake Alexandrina (170°27'E, 43°56'S) and Lake Paringa (169°24'E, 43°43'S). On days 1 and 3 of the experiment, metacercariae were dissected from 5 Lake Alexandrina snails, concentrated in the tip of a fine vinyl tube (diameter 2 mm), and syringed down the throat of a mouse. The mouse was held in a cage that was supported above a plastic aquarium from days 2 to 6 of the experiment. The floor of the cage was constructed from a plastic mesh of sufficient size to allow faecal pellets to pass directly into the experimental aquarium below, thus avoiding desiccation. A second aquarium of the same type was used as a control, into which no faecal pellets were added. Both aquaria (floor dimensions: 70 x 70 cm) were supplied with fresh running water (16 - 18°C), which was maintained at a depth of about 15 cm by using a mesh-covered outlet at that height. [Note: a less invasive method for administering the metacercariae to mice has been developed more recently. Metacercariae can be pipetted on to small bread pieces and fed to mice preconditioned to accept bread as food.]

Snails from each lake (N = 125) were combined in both aquaria on the 13th day of the experiment. Individuals from the two lake populations did not need to be marked for subsequent identification, as they could be separated by distinguishing shell characteristics (Lake Paringa snails have long spines on the outer whorls, whereas those from Lake Alexandrina have smooth shells or shells with short spines). After 3 months, the surviving snails were scored for presence/absence of infection. Infected snails were scored as harbouring either metacercariae (the fully formed encysted stage) or blastocercariae (the unencysted larval stage which precedes the formation of the fully formed metacercarial stage). The results were analysed by a 3-way test of independence.

RESULTS

Higher levels of infection by larval *Microphallus* were found in the aquarium where snails were exposed to the faecal pellets of a mouse harbouring adult *Microphallus* (Fig. 1). A test of conditional independence between treatment and infection, while holding the effects of snail population constant, were highly significant (G = 43.14, df = 2, $P < 0.0001$). This difference between treatments was clearly the result of new infections, which were manifested as unencysted blastocercariae (Fig. 1), and it indicates that the parasite can survive and produce viable, infective eggs in mice.

In addition, a highly significant interdepend-

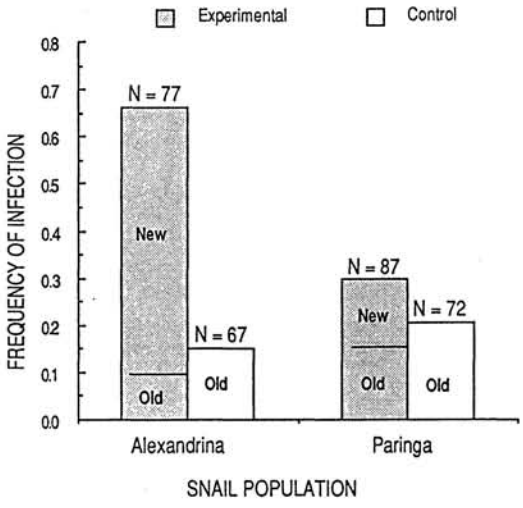

Fig. 1. Results of the infection experiment. Shaded bars show the frequency of infection of surviving *P. antipodarum* in the aquarium in which snails from Lake Alexandrina and Paringa were exposed to the faecal pellets of a mouse, which had been fed the metacercariae of *Microphallus* sp. collected from Lake Alexandrina. "New" refers to snails infected by blastocercariae, and "old" refers to snails infected by fully formed metacercariae. Open bars show the frequency of infection by snails in the control aquarium.

ence among snail population, treatment, and infection was observed ($G = 52.72$, $df = 4$; $P < 0.0001$). This result reflects the higher infection in Lake Alexandrina snails than in Lake Paringa snails in the aquarium where the eggs from the Lake Alexandrina *Microphallus* were added (Fig. 1). A similar result was obtained by comparing the number of "new" infections (blastocercariae present) versus uninfected snails in the experimental aquarium only: Lake Alexandrina snails showed a greater frequency of new infections than Lake Paringa snails ($G = 31.26, df = 1, P < 0.0001$; Fig. 1). Both analyses indicate that Lake Alexandrina snails were more likely to be experimentally infected than Lake Paringa snails.

DISCUSSION

The results of this study clearly demonstrate that mice can be substituted for ducks as the definitive host for *Microphallus* sp., and that this trematode can produce in mice viable eggs that are infective to *Potamopyrgus antipodarum* under laboratory conditions.

The results also show that Lake Alexandrina snails are more likely that Lake Paringa snails to be infected by *Microphallus* collected from Lake Alexandrina. This suggests either that: (1) Lake Alexandrina snails are inherently more susceptible to infection by this trematode, or (2) the trematodes are locally adapted to their snail populations. A reciprocal-transplant experiment using methodology similar to that presented here gave results consistent with the second hypothesis of local adaptation (Lively 1989). Similar indications of greater susceptibility of snails to trematodes collected from the geographic region have been reported for schistosomes (*eg.* Files and Cram 1949, Newton 1953, Richards 1975, Basch 1976, Michelson and DuBois 1978, Chiu *et al.* 1981).

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